Geometrical model for the native-state folds of proteins

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Abstract

We recently introduced a physical model [1, 2] for proteins which incorporates, in an approximate manner, several key features such as the inherent anisotropy of a chain molecule, the geometrical and energetic constraints placed by the hydrogen bonds and sterics, and the role played by hydrophobicity. Within this framework, marginally compact conformations resembling the native state folds of proteins emerge as broad competing minima in the free energy landscape even for a homopolymer. Here we show how the introduction of sequence heterogeneity using a simple scheme of just two types of amino acids, hydrophobic (H) and polar (P), and sequence design allows a selected putative native fold to become the free energy minimum at low temperature. The folding transition exhibits thermodynamic cooperativity, if one neglects the degeneracy between two different low energy conformations sharing the same fold topology.

I. INTRODUCTION

Proteins are well-tailored chain molecules employed by life to store and replicate information, to carry out a dizzying array of functionalities and to provide a molecular basis for natural selection. A protein molecule is a large and complex physical system with many atoms. In addition, the water molecules surrounding the protein play a crucial role in its behavior. At the microscopic level, the laws of quantum mechanics can be used to deduce the interactions but the number of degrees of freedom are far too many for the system to be studied in all its detail. When one attempts to look at the problem in a coarse-grained manner[3] with what one hopes are the essential degrees of freedom, it is very hard to determine what the effective potential energies of interaction are. This situation makes the protein problem particularly daunting and no solution has yet been found. Nevertheless, proteins fold into a limited number[4, 5] of evolutionarily conserved structures[6, 7]. The same fold is able to house many different sequences which have that conformation as their native state and is also employed by nature to perform different biological functions, pointing towards the existence of an underlying simplicity and of a limited number of key principles at work in proteins.

We have recently shown that a simple model which encapsulates a few general attributes common to all polypeptide chains, such as steric constraints[8, 9, 10], hydrogen bonding[11, 12, 13] and hydrophobicity[14], gives rise to the emergent free energy landscape of globular proteins [1, 2]. The relatively few minima in the resulting landscape correspond to distinct putative marginally-compact native-state structures of proteins, which are tertiary assemblies of helices, hairpins and planar sheets. A superior fit[15, 16] of a given protein or sequence of amino acids to one of these pre-determined folds dictates the choice of the topology of its native-state structure. Instead of each sequence shaping its own free energy landscape, we find that the overarching principles of geometry and symmetry determine the menu of possible folds that the sequence can choose from.

Sequence design would favor the appropriate native state structure over the other putative ground states leading to a energy landscape conducive for rapid and reproducible folding of that particular protein. Nature has a choice of 20 amino acids for the design of protein sequences. A pre-sculpted landscape greatly facilitates the design process. Indeed we will show in detail that, within our model, a crude design scheme, which takes into account the hydrophobic (propensity to be buried) and polar (desire to be exposed to the water) character of the amino acids, is sufficient to carry out a successful design of sequences with one of the structures shown in Fig. 1.

II. MODEL AND METHODS

We model a protein as a chain of *identical* amino acids, represented by their C^{α} atoms, lying along the axis of a self-avoiding flexible tube. The preferential parallel placement of nearby tube segments approximately mimics the effects of the anisotropic interaction of hydrogen bonds, while the space needed for the clash-free packing of side chains is approximately captured by the non-zero tube thickness[17, 18, 19]. A tube description places constraints on the radii of circles drawn through both local and non-local triplets of C^{α} positions of a protein native structure[18, 20].

Unlike unconstrained matter for which pairwise interactions suffice, for a chain molecule, it is necessary to define the context of the object that is part of the chain. This is most easily carried out by defining a local Cartesian coordinate system whose three axes are defined by the tangent to the chain at that point, the normal, and the binormal which is perpendicular to both the other two vectors. A study[1, 2] of the experimentally determined native state structures of proteins from the Protein Data Bank reveals that there are clear amino acid aspecific geometrical constraints on the relative orientation of the local coordinate systems due to sterics and also associated with amino acids which form hydrogen bonds with each other. Similar geometrical constraints had already been introduced in off-lattice polymer models [21, 22] in order to model hydrogen bond formation.

The geometrical constraints associated with the formation of hydrogen bonds and with the tube description within the C^{α} representation of our model are described in detail elsewhere[1, 2]. In our representation of the protein backbone, local hydrogen bonds form between C^{α} atoms separated by three along the sequence with an energy defined to be -1 unit, whereas non-local hydrogen bonds are those that form between C^{α} atoms separated by more than 4 along the sequence with an energy of -0.7. This energy difference is based on experimental findings that the local bonds provide more stability to a protein than do the non-local hydrogen bonds[23]. Cooperativity effects[24, 25] are taken into account by adding an energy of -0.3 units when consecutive hydrogen bonds along the sequence are formed. There are two other ingredients in the model: a local bending penalty e_R which is related to the steric hindrance of the amino acid side chains and a pair-wise interaction e_W of the standard type mediated by the water[14]. Note that whereas the geometrical constraints associated with the tube and hydrogen bonds are representative of the typical *aspecific* behavior of the interacting amino acids, the latter properties clearly depend on the *specific* amino acids involved in the interaction.

Monte Carlo simulations have been carried out with pivot and crankshaft moves commonly used in stochastic chain dynamics [26]. A Metropolis procedure is employed with a thermal weight $\exp(-E/T)$, where E is the energy of the conformation and T is the effective temperature.

III. RESULTS AND DISCUSSION

Figure 1 shows the ground state phase diagram obtained from Monte-Carlo computer simulations using the simulated annealing technique[27], along with the corresponding conformations, for a 24 beads homopolymer [1, 2]. The solvent-mediated energy, e_W, and the local bending penalty, e_R, are measured in units of the local hydrogen bond energy. When e_W is sufficiently repulsive (hydrophilic) (and $e_R > 0.3$ in the phase diagram), one obtains a swollen phase with very few contacts between the C^α atoms. When e_W is sufficiently attractive, one finds a very compact, globular phase with featureless ground states with a high number of contacts.

Between these two phases (and in the vicinity of the swollen phase), a marginally compact phase emerges (the interactions barely stabilize the ordered phase) with distinct structures including a single helix, a bundle of two helices, a helix formed by β-strands, a β-hairpin, three-stranded β-sheets with two distinct topologies and a β-barrel like conformation. These structures are the stable ground states in different parts of the phase diagram. Furthermore, other conformations, closely resembling distinct super-secondary arrangements observed in proteins[4], also shown in Fig. 1, are found to be competitive local minima, whose stability can be enhanced, as we will see, by sequence design after heterogeneity is introduced by means of, for example, non-uniform values of curvature energy penalties for single amino acids and hydrophobic interactions for amino acid pairs. Note that while there is a remarkable similarity between the structures that we obtain and protein folds, our simplified coarse-grained model is not as accurate as an all-atom representation of the poly-peptide chain in capturing features such as the packing of amino acid side chains.

The common belief in the field of proteins is that given a sequence of amino acids, with all the attendant details of the side chains and the surrounding water, one obtains a funnel-like landscape with the minimum corresponding to its native state structure. Each protein is characterized by its own landscape. In this scenario, the protein sequence is all-important and the protein folding problem, besides becoming tremendously complex, needs to be attacked on a protein-by-protein basis.

In contrast, our model calculations show that the large number of common attributes of globular proteins[18, 28] reflect a deeper underlying unity in their behavior. At odds with conventional belief, the gross features of the energy landscape of proteins result from the amino acid aspecific common features of all proteins, as is clearly established by the fact that different putative native structures are found to be competing minima for the same homopolymeric chain.

The protein energy landscape is *(pre)sculpted* by general considerations of geometry and symmetry (Fig. 2), which are the essential ingredients in our model. Our unified framework suggests that the protein energy landscape ought to have around a thousand of broad minima corresponding to putative native state structures. The key point is that for each of these minima the desirable funnel-like behavior is already achieved at the homopolymer level *in the marginally compact part of the phase diagram.* The self-tuning of two key length scales, the thickness of the tube and the interaction range, to be comparable to each other and the interplay of the three energy scales, hydrophobic, hydrogen bond, and bending energy, in such a way as to stabilize marginally compact structures, also provide the close cooperation between energy gain and entropy loss needed for the sculpting of a funneled energy landscape. At the same time, relatively small changes in the parameters e_W and e_R lead to significant differences in the emergent ground state structure, underscoring the sensitive role played by chemical heterogeneity in selecting from the menu of native state folds.

The introduction of sequence heterogeneity at the level of differantiating hydropohobic (H) and polar (P) residues and a crude designe scheme based on a common sense choice of the hydrophobicity profile for the sequence suffices to ensure that the designed sequence would fold into a desired fold selected from the predetermined menu. For example, the β-α-β motif shown as (j) in Fig. 1 (which is a local energy minimum for a homopolymer) can be stabilized into a global energy minimum for the sequence HPHHHPPPPHHPPHHPPPPHHHPP, with $e_W = -0.4$ for HH contacts and $e_W = 0$ for other contacts, and $e_R = 0.3$ for all residues.

We have studied the thermodynamic properties of the folding transition for this case. The contour plots at different temperatures (above and at the folding transition temperature) of the effective free energy are shown in Fig. 3(a) and 3(b) as a function of the total energy of the chain and its root mean squared deviation (RMSD) from the β-α-β conformation obtained as a local minimum in the homopolymer case. The folding transition temperature was identified at $T = 0.18$ from the peak of the specific heat curve (data not shown).

The free energy landscape is not too smooth at low temperatures, but it can be roughly be described as a three state landscape. Above the folding transition there is one broad minimum, corresponding to the denatured state ensemble. The two clearly distinct minima which dominate the free energy landscape below the folding transition correspond to the designed conformation (in the case of the lower RMSD) and to a different conformation (with higher RMSD). Both structures share the same number of HH contacts as well as local, non-local, and cooperative hydrogen bonds, differing just in the way the two strands are connected with the helix (see Figure 3(c) and 3(d)). Note that both minima are consistently characterized by roughly the same energy.

This degeneracy is due to the lack of detail of the model and to the crudeness of the design scheme utilized here, since the hydrophobic core is well formed in both cases (see Fig. 3(c) and 3(d)). Indeed, if one groups both conformations within the same fold classification, this is a clear example of how, in the presence of a presculpted energy landscape, it is relatively easy to design a sequence with the ability to fold cooperatively into a given fold, as real small globular proteins do.

IV. CONCLUSIONS

In summary, within a simple, yet realistic, framework, we have shown [1, 2] that protein native-state structures can arise from considerations of symmetry and geometry associated with the polypeptide chain. The sculpting of the free energy landscape with relatively few broad minima is consistent with the fact that proteins can be designed to enable rapid folding to their native states. Here we have shown that by introducing heterogeneity within the simplest hydrophobic-polar scheme, it is straightforward to design a sequence that is able to fold cooperatively into one of the presculpted minima in the energy landscape.

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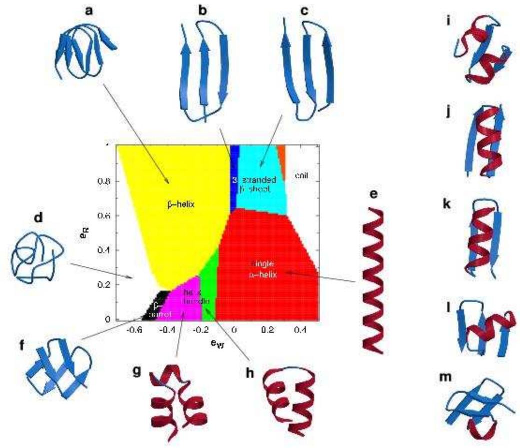


FIG. 1: Phase diagram of ground state conformations. The ground state conformations were obtained by means of Monte-Carlo simulations of chains of 24 C^{α} atoms. e_R and e_W denote the bending energy penalty and the solvent mediated interaction energy respectively. Over 600 distinct local minima were obtained in our simulations in different parts of parameter space starting from a randomly generated initial conformation. The temperature is set initially at a high value and then decreased gradually to zero. (a), (b), (c), (e), (f), (g), (h) are the Molscript representation of the ground state conformations which are found in different parts of the parameter space as indicated by the arrows. The helices and strands are assigned when local or non-local hydrogen bonds are formed according to the rules employed within our model[1]. Conformations (i), (j), (k), (l), (m) are competitive local minima. In the orange phase, the ground state is a 2-stranded β-hairpin (not shown). Two distinct topologies of a 3-stranded β-sheet (dark and light blue phases) are found corresponding to conformations shown in conformations (b) and (c) respectively. The white region in the left of the phase diagram has large attractive values of e_W and the ground state conformations are compact globular structures with little amount of secondary structures. At even lower values of e_W, the ground states exhibit a crystalline order induced by hard sphere packing considerations[29] and not by hydrogen bonding (conformation (d)).

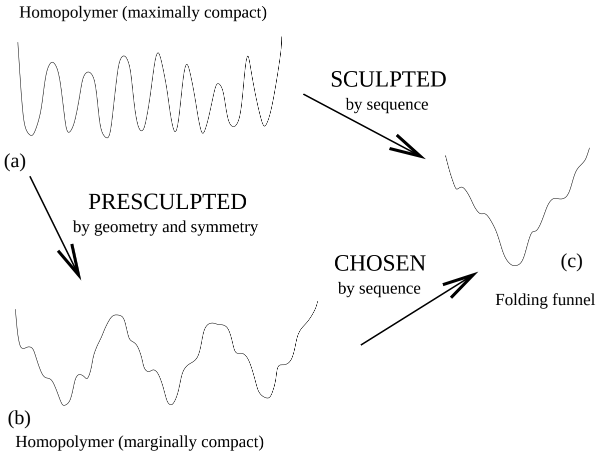
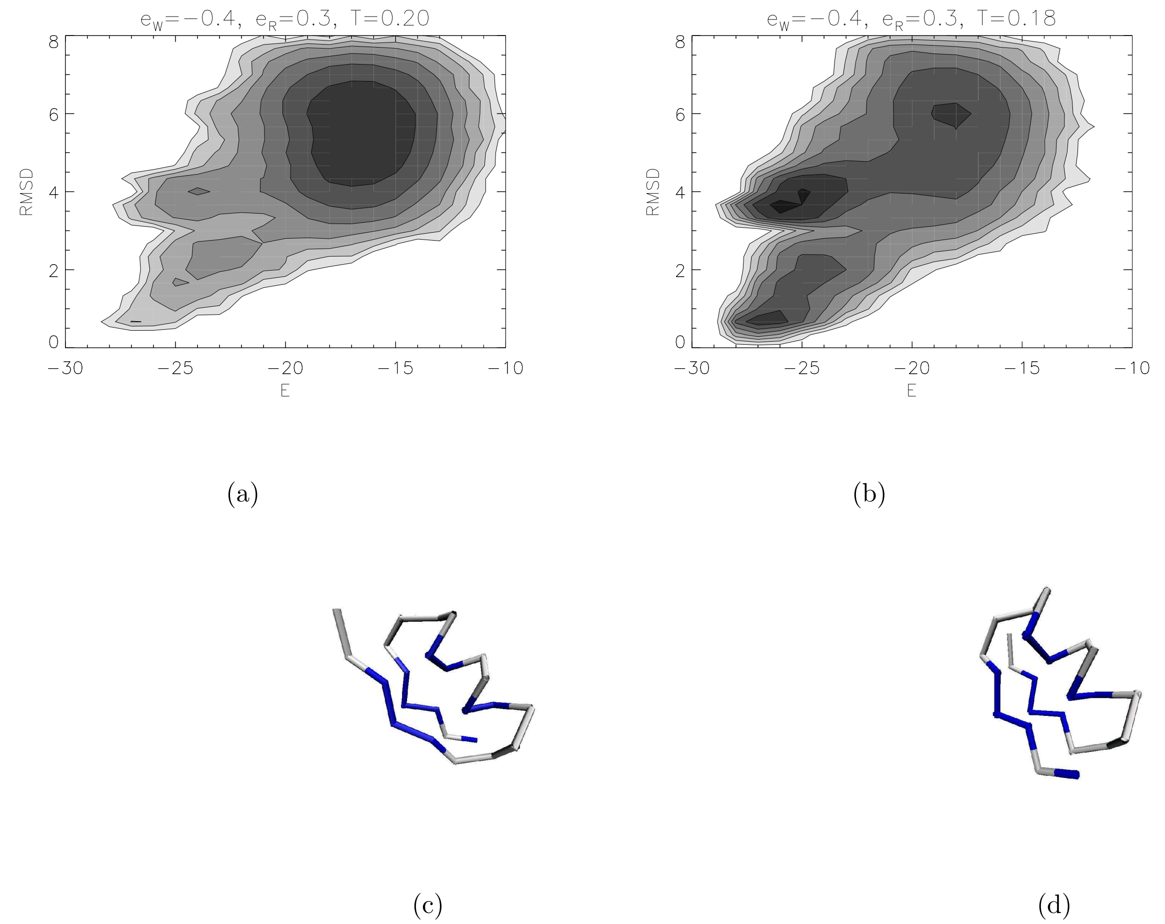


FIG. 2: Simplified one dimensional sketches of energy landscape. The quantity plotted on the horizontal axis schematically represents a distance between different conformations in the phase space and the barriers in the plots indicate the energy needed by the chain in order to travel between two neighboring local minima. (a) Rugged energy landscape for a homopolymer chain with an attractive potential promoting compaction as, e.g., in a string and beads model. There are many distinct maximally compact ground state conformations with roughly the same energy, separated by high energy barriers (the degeneracy of ground state energies would be exact in the case of both lattice models and off-lattice models with discontinuous square-well potentials). (b) Presculpted energy landscape for a homopolymer chain in the marginally compact phase. The number of minima is greatly reduced and the width of their basin increased by the introduction of geometrical constraints. (c) Funnel energy landscape for a protein sequence. As folding proceeds from the top to the bottom of the funnel, its width, a measure of the entropy of the chain, decreases cooperatively with the energy gain. Such a distinctive feature, crucial for fast and reproducible folding, arises from careful sequence design in models whose homopolymer energy landscape is similar to (a). In contrast, funnel-like properties already result from considerations of geometry and symmetry in the marginally compact phase (b), thereby making the goals of the design procedure the relatively easy task of stabilization of one of the pre-sculpted funnels followed by the more refined task of fine-tuning the putative interactions of the protein with other proteins and ligands.



(a)

(b)

(c)

(d)

FIG. 3: Contour plots of the effective free energy (a) at high temperature ($T = 0.20$) and (b) at the folding transition temperature $T_f = 0.18$ for the HPHHHPPPPHHPPHHPPPPHHHPP 24-residue chain, with $e_W = -0.4$ for HH interacions, $e_W = 0$ for other interactions, and $e_R = 0.3$. The effective free energy, defined as $F(E, RMSD) = -\ln N(E, RMSD)$, is obtained as a function of the energy E and the root mean squared deviation $RMSD$ from the 'reference' conformation shown in Fig. 1(j), from the histogram $N(E, RMSD)$ collected in equilibrium Monte-Carlo simulations at constant temperature. The spacing between consecutive levels in each contour plot is 1 and corresponds to a free energy difference of $k_B\tilde{T}$, where $\tilde{T}$ is the temperature in physical units. The darker the color, the lower the free energy value. There is just one free energy minimum corresponding to the denatured state at a temperature higher than the folding transition temperature (Panel (a)) whereas one can discern the existence of three distinct minima at the folding transition temperature (Panel (b)). The two conformations corresponding to the two low energy minima are shown in Panel (c) (higher RMSD) and (d) (lower RMSD). Hydrophobic (polar) residues are shown in dark (light) grey